having been gradually accomplished after birth, gross bulbar disturbance, blindness, faulty muscle action, and coarse atrophic disorder have not been produced, and hence remain unmentioned as ordinary consequences in such cases.

Blindness from deprivation (postnatal causes), as in the wide-world known case of Laura Bridgman, which on autopsy was found to be associated with optic nerve and optic tract atrophy and thinning of the gray matter of the occipital cortex, is also a subject for discussion elsewhere.

ON THE CONTINUITY OF PROTOPLASM.

BY HENRY KRAEMER, PH.D.

(Plates XXI and XXII.)

(*Read April 4, 1902.*)

While Schleiden[1] conceived each cell to have an independent existence, Hofmeister[2] contended that the protoplasts of contiguous cells are united, forming a higher unity; that is, one synplast. In later years both Sachs[3] and Strasburger[4] have supported the view of Hofmeister. And even so great an authority as Nägeli[5] expressed the view that neighboring plant cells are united by means of threads of protoplasm in much the same manner as in the sieve tubes first described by Hartig[6] some thirty years before.

In 1878 Thuret and Bornet[7] first called attention to the fact that in certain of the Florideæ the contents of certain of the cells of the trichophore and carpogonium are directly connected by means of pores. Fromann[8] appears first to have called attention to the direct connection of protoplasm in the higher plants, in the epidermal and parenchyma cells in the leaves of Rhododendron and Dracena. While Tangl[9] was preceded by these several investigators, the establishment of the view that there is a continuity of protoplasm is due for the most part to his researches. On treating dry sections of the endosperm of *Strychnos Nux vomica* with dilute iodine solutions, he observed a distinct lamellation of the cell wall as well as the formation of yellowish striæ, which latter he conceived to be plasma threads connecting the different cells. The appearance thus produced he compares to the structure of the sieve tubes, but in speaking of the contents of the latter, he states that

they can hardly be considered to be in the nature of protoplasm, and substantiates this statement by quoting from De Bary and Sachs.

A few years later Gardiner,[10] while working in the laboratory of Sachs on certain sensitive plants, observed by the use of sulphuric acid or chlor-zinc-iodide and Hofmann's blue or methylene blue, colored striæ in the walls of certain of the cells, which he considered to be in the nature of threads of protoplasm. A number of other workers have also considered this subject, using a similar technique to that of Gardiner, confirming his observations and extending the number of species showing a continuity of protoplasm.

The results obtained by these investigators tend to show that there are two kinds of continuity of protoplasm, one through openings in the pores which apparently occur in the larger number of cases, and another in which the threads of protoplasm extend through walls in which there are no pores. Several investigators[11] even go so far as to express the view that probably every cell is connected with its neighboring cells by protoplasmic threads.

That there is a continuity of protoplasm has become almost a fundamental principle in botany, it being considered necessary in the transmission of irritation currents and in the distribution of protoplasm and such bodies as starch grains and oil globules, intact and quickly from cell to cell.

While fully cognizant of the plausible arguments which have been advanced in favor of the continuity of protoplasm, and, furthermore, not desiring to consider the subject theoretically, by the discussion of certain facts in regard to solution, osmosis, the ascent of sap, and other physical phenomena that might more favorably assist the plant in its various functions than a protoplasmic connection between the cells, the author presents herewith some of the results of his studies on the structure of the starch grain and cell wall, in the belief that they will throw some additional light on the subject under consideration.

Suffice it to say that these results seem to offer a different explanation for the phenomena observed by the investigators already mentioned, in their studies on the continuity of protoplasm. In other words, the appearances described by these authors as indicating a continuity of protoplasm are due to a peculiarity in the structure of the cell wall, which is made manifest by the reagents

employed and which bears an analogy to the structure of the starch grain.

In the author's studies on the starch grain, the following observations have been made:

(1) The illustrations of potato starch in the various text-books show two kinds of grains, one with the point of growth and the alternate lamellæ light in color, as figured by Sachs (Plate XXI, Fig. 1), and the other with the point of growth and alternate lamellæ dark, as figured by Strasburger (Fig. 2). This appearance, however, is not due to a difference in the grains, but is brought about by the manner of focusing on them. In the figure given by Strasburger the lamellæ are viewed from above, while in the figure of Sachs the view is from below.

(2) On treating the starch grain with water at different temperatures and a number of reagents,* a radiating crystal-like structure is observed in the successive layers (Fig. 5). This crystalline structure appears to be most pronounced in the layers alternating with the point of growth, and is succeeded by the formation of a number of clefts or fissures (Figs. 6 and 7). In potato starch these clefts are more or less feather-like in appearance, and extend from the point of growth through the middle of the successive layers to the periphery of the grain. In wheat starch the fissures extend radially from near the point of growth to near the periphery.

(3) On treating starch grains with weak aqueous solutions of various aniline dyes, as gentian violet, eosin and safranin, it is observed that the layers which are less crystalline or colloidal in character take up the stains (Figs. 3, 4 and 7). The various clefts and fissures produced in the grains behave toward staining reagents much like the colloidal layers, and they are probably the tracts or channels through which liquids are distributed throughout the grain.

(4) We further find that these two kinds of layers behave differ-

* The reagents used were the following: (1) Chromic acid solution (5 to 15 per cent.); (2) Calcium nitrate solution (5 to 30 per cent.); (3) Potassium hydrate solution (one-tenth of 1 per cent.); (4) Sulphuric acid (10 per cent.); (5) Silver nitrate solution (2 per cent.); (6) Sodium acetate solution (50 per cent.); (7) Potassium nitrate solution (saturated); (8) Potassium phosphate solution (saturated); (9) Hydrochloric acid (5 per cent.); (10) Potassium iodide solution (1 to 10 per cent.); (11) Tannic acid solution (5 to 15 per cent.); (12) Saliva; (13) Taka-diastase (saturated solution); (14) Chlor-zinc-iodide solution; (15) Chloral iodine solution and iodine water, equal parts.

ently toward iodine; the one rich in crystalloidal substance becomes blue with iodine, whereas the other is not affected by this reagent.

In the studies of the author on the structure of the cell wall, the following observations tending to show an analogy to the starch grain have been made:

(1) A similar layering of the cell wall, known as stratification and striation, is readily observable in the walls of endosperm cells as well as those cells impregnated more or less with mucilage, lignin, cutin, suberin and allied substances. In some cases the use of reagents, as acids and alkalies, may be necessary to bring out this structure (Fig. 8). While it is not always easy to determine the nature of the successive layers in the wall, still the structure seems to correspond in the main to that of the starch grain, the middle lamella of the cell corresponding to the point of growth.

(2) The same kind of reagents, but in stronger solutions, may be used to bring out the crystalline or spherite structure in the walls of thickened parenchyma cells, as endosperm (Plate XXII, Figs. 9 and 13), or lignified cells, as stone cells. In cases where the cell wall has been metamorphosed into mucilage, simple treatment with water, as has also been shown to be the case with the starch grain, is sufficient to bring out this structure.

(3) The differentiation of the layers of the cell wall by the use of aniline stains,* has not as yet been attended with any marked degree of success. The use of swelling reagents, as sulphuric acid, in conjunction with a stain, has, however, produced more or less interrupted striæ resembling the clefts and fissures in the starch

* The methods involving the use of aniline stains in the study of the cell wall are the same as those used in the study of the continuity of protoplasm, and embody the three operations of fixing, swelling and staining, between each of which operations the sections are washed quickly and with large quantities of water. Fixing is usually accomplished by the use of aqueous iodine solutions (.5 per cent. of iodine and .5 to 1 per cent. of potassium iodide); alcohol, osmic and picric acids may also be employed. The swelling of the specimens is effected by the use of dilute sulphuric acid (25 to 75 per cent.), iodine being sometimes added to the sulphuric acid solution; chlor-zinc-iodide and solutions of the alkalies are also employed for this purpose. The stains mostly employed are 5 per cent. aqueous solutions of gentian violet, eosin or safranin, these being used in connection with the swelling agents mentioned above. The time required for each operation is usually from five to ten minutes, but when chlor-zinc-iodide is used twelve hours may be required for the swelling.

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grain. In the case of Nux vomica, solutions of potassium iodide and iodine produce yellowish-brown striæ in fresh sections (Fig. 13), closely resembling in form those produced by aniline stains (Fig. 14), and which were considered by Tangl as being protoplasmic threads, but which are probably due to the precipitation of an alkaloidal salt in the clefts or fissures in the wall.*

(4) The two kinds of layers behave differently toward chlor-zinc-iodide; the one next to the middle lamella and those alternating with it are colored blue, while the others are but slightly affected.

The observations and comparisons herewith presented lead to the following interpretations:

(1) The starch grain, as also the cell wall, is made up of alternate lamellæ of colloidal and crystalloidal substances.

(2) Physically, the structure of the starch grain and cell wall are quite similar, although chemically different; the preponderating substance in the starch grain being granulose, while in the cell wall the fundamental substance is cellulose, which may preponderate or exist in varying proportions.

(3) The crystalloidal layer in the starch grain, consisting chiefly of granulose, is colored blue with iodine or chlor-zinc-iodide, whereas in the cell wall this layer, consisting chiefly of cellulose, is colored blue only with chlor-zinc-iodide.

(4) The colloidal layers in both the starch grain and cell wall take up and hold various aniline dyes, the layers being, however, more clearly defined in the starch grain, particularly potato starch.

(5) In starch grains as in cell walls, there are radial clefts or colloidal areas which under certain conditions also take up and hold various aniline stains.

(6) The plastid at the periphery of the starch grain may be compared to the protoplasm of the plant cell, each contributing to the growth of successive new layers. In the cell wall the mode of growth is centripetal, whereas in the starch grain it is centrifugal.

The peculiar bi-convex arrangement of the groups of striæ between contiguous cells in the Nux vomica and vegetable ivory is rather suggestive of fundamental lines of development corresponding to chromatin threads, although they may be modifications of the wall

* This may explain why the iodine method alone has not met with any success save in the case of fresh sections of Nux vomica.

and represent tracts or channels through which liquids are distributed from cell to cell.

Furthermore, attention should be directed to the fact that the preparations of both the starch grain and cell wall showing the colored lamellæ and striæ, as already described, are permanent only in Canada balsam and are ephemeral in glycerin or glycerin jelly.

Finally, it may be stated that all authors since the appearance of Gardiner's work* have fallen into the error of supposing that a certain aniline dye could be regarded as a differential stain for protoplasm, whereas the fact of the matter is that many colloidal carbohydrates, as mucilage and pectin, and oils and other substances as well, take up these stains. And in this connection we may ask, If the substance in the cell wall which takes up the stain is protoplasm, what is it in the starch grain?

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Explanation of Plates.

Plate XXI.

Fig. 1. Potato starch grain with point of growth and alternate lamellæ light in color.

* Gardiner states that "All experiments made with the view of attempting to detect the presence of protoplasmic filaments in the cell wall when the cell was normal and intact met with but little success, so that in investigating the subject of protoplasmic continuity the method of swelling the cell wall and subsequently staining with a dye which was found to especially stain the protoplasm was adopted."

Fig. 2. Potato starch grain with point of growth and alternate lamellæ dark.

Fig. 3. Potato starch grain treated with aqueous solution of gentian violet.

Fig. 4. Potato starch grain treated with gentian violet and showing crystalloidal structure in alternate lamellæ.

Fig. 5. Wheat starch grain treated with water at 60° C., or with chromic acid and other reagents (see footnote *).

Fig. 6. Wheat starch grain treated with water at a temperature of 65° C., or with the reagents mentioned in footnote *, but for a longer time.

Fig. 7. Wheat starch grain treated with aqueous safranin solution.

Fig. 8. Cells of the endosperm of Date seed (*Phœnix dactylifera*), the one normal and the other showing the stratification of the wall after treatment with chlor-zinc-iodide.

Plate XXII.

Fig. 9. Cell of vegetable ivory (*Phytelephas macrocarpa*), showing lamellation and crystalline structure in the wall after treatment with chlor-zinc-iodide, clove oil, chromic acid or other reagents.

Fig. 10. Pore of vegetable ivory showing cleft in middle lamella.

Figs. 11 and 12. Pores of vegetable ivory showing striæ between neighboring cells after treatment with sulphuric acid and gentian violet.

Fig. 13. Cells of endosperm of the seed of *Strychnos Nux vomica* after treatment with iodine solution.

Fig. 14. Cell of endosperm of seed of Nux vomica treated with sulphuric acid and gentian violet.